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<b>14. ABSTRACT</b> Transposon mutagenesis was used to make mutants in Shewanella oneidensis strain MR-1. Two separate genetic screens were performed: the first identified mutants that are defective in their ability to reduce AQDS (a proxy for humic acid), the second identified mutants that are defective in their ability to make biofilms on steel chips. The mutants were subjected to a variety of tests to verify their phenotypes and further characterize their properties. The site of insertion of the transposon was determined. Select mutants defective in either humic acid reduction or biofilm formation were compared with respect to their ability to promote the corrosion of mild steel using electrochemical impedance spectroscopy. The principle results from this study were: 1.) the discovery that bacteria produce their own extracellular electron shuttles that can transfer electrons to iron minerals, and 2.) that iron reduction can protect steel from corrosion.					
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## FINAL REPORT

**GRANT NUMBER:** N00014-00-1-0083

**PRINCIPAL INVESTIGATOR:** Dr. Dianne Newman

**GRANT TITLE:** The Molecular Basis of Humic Acid Reduction and its Role in Microbiologically Influenced Corrosion

**AWARD PERIOD:** 1/1/2000-8/30/20002

**OBJECTIVE:** To identify the mechanisms of indirect mineral dissolution (mediated by microbial reduction of humic acid) and direct biofilm formation on minerals.

**APPROACH:** Transposon mutagenesis was used to make mutants in *Shewanella oneidensis* strain MR-1. Two separate genetic screens were performed: the first identified mutants that were defective in their ability to reduce AQDS (a proxy for humic acid), the second identified mutants that were defective in their ability to make biofilms on steel chips. Candidate mutants were subjected to a variety of tests to verify their phenotypes and further characterize their properties. The site of insertion of the transposon was determined. Depending on the nature of the mutation, individual mutants were studied further. Select mutants defective in either humic acid reduction or biofilm formation were compared with respect to their ability to promote the corrosion of mild steel using electrochemical impedance spectroscopy.

### ACCOMPLISHMENTS:

Our genetic screen with AQDS revealed two different mutant classes, each of which taught us something new. The first mutant class (exemplified by the *menC* mutant) was completely defective in electron transfer to AQDS. We found that it could be complemented by an extracellular diffusible factor excreted by the wild-type, causing us to propose that microbiologically-produced small electron shuttling molecules play a role in bacterial mineral respiration. This idea has since been verified by Derek Lovley's (UMass) and John Zachara's (PNNL) labs, who have direct evidence that iron reduction by *Shewanella* can proceed in a delocalized fashion, without requiring direct contact between microbes and minerals. Our second mutant class (exemplified by the *tolC* mutant) was defective in transport of AQDS out of the cell. Because the TolC pathway is also required for the excretion of certain types of redox-active antibiotics, we reasoned that there might be a hitherto unrecognized link between the two. Switching over to work with *Pseudomonas* bacteria, we were able to show that representatives of the numerous multicolored phenazine pigments produced by these bacteria can function as electron shuttles to minerals and be exchanged between species. These molecules not only affect the rate of mineral transformation by known metal-reducing species, such as *S. oneidensis*, but they also enable a variety of bacteria not commonly thought to be metal-

reducers to contribute to this process. This suggests a new role for a broad group of secondary metabolites and demonstrates that the exchange of redox active "antibiotics" is likely to have an important geochemical impact. We are currently writing this up for publication (ME Hernandez, A. Kappler and DK Newman.)

Our genetic screen to identify mutants defective in biofilm formation allowed us to determine that biofilm formation by *Shewanella oneidensis*, *per se*, was overrated with respect to its effect on corrosion. In collaboration with Dr. Florian Mansfeld's group at USC, we were able to use electrochemical impedance spectroscopy to quantify the influence of *Shewanella* on corrosion. Counter to the prevailing theories about microbiologically-influenced corrosion (MIC), our results showed that *Shewanella* can protect steel from corrosion through iron reduction (under conditions where ferrous iron can accumulate in solution, and thereby serve as a reductant for oxygen so that it is unable to oxidize the steel surface). This result has garnered much attention, and we currently have a review article in press for *Applied Microbiology and Biotechnology* on the general phenomenon of corrosion inhibition as mediated by iron-reducing bacteria.

**CONCLUSIONS:** Our studies have revealed that bacteria release extracellular electron shuttles that can be used to reduce minerals, and that microbial iron respiration can protect steel from corrosion.

**SIGNIFICANCE:** Two important findings have come out of our work. The first is that there appears to be a relationship between electron shuttling molecules and certain classes of antibiotics. The second is that we have shown that iron-reducing biofilms may protect steel from corrosion. We believe both of these findings will have a significant impact. The first, because it may change our thinking about the evolutionary basis for antibiotics; the second, because it may have practical consequences for how people treat MIC.

**PATENT INFORMATION:** We asked Caltech to apply for a provisional patent for the idea that iron-reducing biofilms may protect steel from corrosion, but have since decided not to follow up on this idea.

**AWARD INFORMATION:** ONR Young Investigator award, Packard Fellowship from the Packard Foundation, Invited speaker at the "Frontiers of Engineering" 2003 meeting, National Academy of Engineering.

## REFEREED PUBLICATIONS

AK Lee and DK Newman. Microbial iron respiration: impacts for corrosion processes, *Appl. Microbiol. Biotechnol.* [invited review], *in press*

D.K. Newman and J.F. Banfield (2002) Geomicrobiology: how molecular scale interactions underpin biogeochemical systems. *Science*, 296:1071-1077.

J. B. Shyu, D.P. Lies and D.K. Newman (2002) Protective role of tolC in the efflux of the electron shuttle, anthraquinone-2,6-disulphonate (AQDS), *J. Bacteriol.*, 184:1806-1810.

M. Dubiel, S. Hsu, C.C. Chien, F. Mansfeld and D.K. Newman (2002) Microbial iron respiration can protect steel from corrosion, *Appl. Environ. Microbiol.*, 68:1440-1445.

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M.E. Hernandez and D.K. Newman (2001) Extracellular Electron Transfer, *Cellular and Molecular Life Sciences*, 58:1562-1571.

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